

Relationships between Soybean Seed Cell Wall Polysaccharides, Yield, and Seed Traits

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ABSTRACT

Seed cell wall polysaccharides (CWPs) represent a significant portion of seed dry matter (DM) in soybean [*Glycine max* (L.) Merr.]. To further investigate the role of seed CWP in determining seed traits, relationships were examined among monosaccharide constituents of CWP and between CWP, yield, maturity, seed weight, and protein and oil content using principal component (PC) analysis in 13 Minnesota-adapted cultivars. Monosaccharide data were used to form PCs. Principal Component 1 mainly described pectin polysaccharides composed of rhamnose and galactose and was negatively correlated with the sum of protein and oil content (protein + oil) ($r = -0.58$). Cellulose, hemicellulose, and arabinose-containing polysaccharides as described by PC2 were negatively correlated with yield ($r = -0.59$), suggesting lower seed content in these CWP relates to an increase in yield. Seed arabinose:galactose (Ara:Gal) ratio was negatively correlated with yield and maturity and may be an indicator of the average stage of seed cell wall development for a cultivar at maturity. Principal Components 2, 3, and 4 combined to form a stepwise regression equation ($R^2 = 0.69$) for seed weight with PC4 providing the most weight in the equation. This equation implicates linear and backbone polysaccharides as being most important in relation to seed weight. Thus, individual polysaccharide types were correlated with either yield, protein + oil, or seed weight. Lower seed content of CWP was correlated with beneficial changes in seed traits, suggesting that reducing CWP content may provide an additional seed trait improvement.

IN SOYBEAN SEED, CWPs represent a significant portion of DM (Daveby and Aman, 1993; Stombaugh et al., 2000). Cell wall polysaccharides are arrays of polymers forming complex matrices (Carpita and Gibeau, 1993) with individual polysaccharides, such as rhamnogalacturonans or xyloglucans, being described and quantified by their linkages and monosaccharide composition (Aspinall et al., 1967). However, the matrix formed from these polymers makes quantitation of individual polysaccharides difficult. Studies have compared cultivars and species using methods that quantify monosaccharides after hydrolyzing the polysaccharides (Champ et al., 1986; Daveby and Aman, 1993; Theander et al., 1995). This tends to conceal some relationships that result from different types of polysaccharides having some monosaccharide constituents in common. In soybean seed, eight monosaccharides from CWP were quantified and their concentrations were found to be correlated to various degrees (Stombaugh et al., 2000). By using additional statistical analyses, the correlations among monosaccharides may be used to increase our understanding of how CWP in soybean seed are interre-

lated and how this interrelationship affects other traits as they vary across cultivars. Principal component analysis (PCA) in particular may be useful for defining how monosaccharides interrelate by deriving clusters which may be related to types of cell wall polysaccharides. These clusters in turn may be used to examine the relationships between CWP, yield, and seed traits.

Principal component analysis reduces interrelated variables into fewer orthogonal components (Chatterjee and Price, 1977; Stevens, 1986). While PCs can be difficult to interpret, their analyses provide a method for observing how multiple input variables interrelate. These interrelationships may not be easily observed using simple correlations; however, PCA clusters similar variation into groups. The magnitude and direction of the value assigned to variables within each PC describes the relationship between variables. Principal component analysis was used in cotton to study genotypic variation in agronomic and fiber traits (Brown, 1991). Twenty fiber and seed traits were distilled into three PCs that accounted for 60 to 70% of the variation. Brown (1991) provided a graphical representation of how multiple genotypic traits could be used to show how cultivars were interrelated by plotting the PC values for each of the cultivars on three-dimensional axes. Cell wall mutants were identified from a mutagenized *Arabidopsis* population by using PCA of Fourier transformed infrared spectra (Chen et al., 1998). Stem cell wall composition in maize was divided into PCs and used to develop regression equations for predicting stem degradability (Jung and Buxton, 1994). In soybean seed, CWP are a composite of seed coat and embryo polysaccharides (Stombaugh et al., 2000). The seed coat CWP are primarily cellulose and hemicellulose, and the cotyledon CWP are primarily pectin (Stombaugh et al., 2000). Principal component analysis provides a means to analyze complex mixtures such as seed CWP.

Soybean CWP were negatively correlated with the sum of seed protein and oil content (Stombaugh et al., 2000). Yet, soybean seed yield can be negatively correlated with protein and seed weight, and positively correlated with maturity (Mansur et al., 1996). Some negative correlations among seed constituents and seed traits indicate that changing some constituents can be counterproductive to advancement of other traits. Selections have been made to reduce or eliminate the negative correlation between protein and yield (Cober and Voldeng, 2000). In seed, CWP are a potential source of carbon that may be diverted into protein and oil by selection of soybeans with reduced levels of CWP. However, since protein has been negatively correlated with yield (Mansur et al., 1996), CWP may be negatively

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Abbreviations: Ara:Gal, arabinose:galactose; CWP, cell wall polysaccharides; DM, dry matter; PC, principal component; PCA, principal component analysis.

correlated with yield as well. Therefore, information on the relationships between seed CWP, yield, and other seed traits will aid in determining the usefulness of selecting for lower seed CWP to produce improved soybean cultivars.

The objectives of this study were to investigate the relationships among monosaccharide constituents of CWP and between CWP, yield, and seed traits as they varied in a population of 13 cultivars adapted to Minnesota. These relationships were described using PCA in conjunction with stepwise regression to provide a detailed description of soybean seed CWP and to determine which CWP were related to yield, maturity, protein, oil, protein + oil, and seed weight. The analysis of these relationships provides a framework for future experiments to investigate the association between CWP, yield, and seed traits in soybean.

MATERIALS AND METHODS

Soybeans of Maturity Groups 00 to I ('Bert', 'Council', 'Faribault', 'Glacier', 'Granite', 'Hendricks', 'Kato', 'Lambert', 'McCall', 'Ozzie', 'Parker', 'Proto', and 'Toyopro') were grown in 1995 and 1996 at Lamberton, Waseca, Rosemont, and Becker, MN, as described previously (Stombaugh et al., 2000). The cultivars were grown in standard yield plots and managed for uniform yield evaluations. Seed samples were taken from the yield trials for analysis. Analysis of CWP, pectin, seed weight, protein, and oil in whole seeds was described previously (Stombaugh et al., 2000). Cell wall polysaccharides were quantified by the Uppsala total dietary fiber method for neutral monosaccharides and the Ahmed and Lavitch (1977) method for uronic acid quantitation. For statistical use, CWP was defined and used as previously as the sum of the eight monosaccharides, and pectin was defined as the sum of rhamnose, fucose, arabinose, galactose, and uronic acids (Stombaugh et al., 2000). Variation among monosaccharides was predominately within genotype, year, or location. For xylose, rhamnose, and galactose, there was some genotype \times year variation, but it was a small part of the total variation (Stombaugh et al., 2000).

Yield was measured from an area of 1.5 m by 2.6 m and calculated as kilograms per hectare. Maturity was recorded as days after 1 September when 95% of the pods had reached their mature color.

Principal components were calculated using the SAS, version 8, factor procedure with the PC method using a correlation matrix (SAS Institute, 2000). To study genotypic variation and reduce variation from other sources, data from eight monosaccharides that constituted CWP quantification were averaged across 2 yr and four locations for 13 cultivars and used for PCA and in correlations. Correlations and stepwise regressions ($F = 4.0$ to enter and 4.0 to leave) were calculated using Statistix 4.0 (Analytical Software, 1992). Arabinose and galactose content (g kg^{-1} DM) were used to calculate Ara:Gal ratios. These ratios were averaged across years and locations to obtain genotypic averages for use in correlations.

RESULTS AND DISCUSSION

Principal Component Analysis of Seed Cell Wall Polysaccharide

Principal components were calculated using eight monosaccharides that constitute CWP to describe the varia-

tion within seed monosaccharide constituents of CWP from 13 soybean cultivars averaged across two years and four locations (Table 1). While signs were assigned to monosaccharides to make the PCs orthogonal, the magnitude and direction of a factor loading within a PC determines the importance and relationship between variables within a component (Stevens, 1986; Chatterjee and Price, 1977). Principal Component 1 accounted for 38.3% of the variation in CWP and was positive for galactose, rhamnose, and fucose, and negative for mannose (Table 1). Principal Component 2 accounted for 19.5% of the variation and was positive for arabinose and glucose. Principal Component 3 accounted for 16.2% of the variation and was negative for xylose and positive for glucose and uronic acids. Principal Component 4 accounted for 9.1% of the variation and was negative for uronic acids. Together, these components accounted for 83.1% of the monosaccharide variation within CWP.

Galactose, rhamnose, fucose, and mannose were the major monosaccharides described in PC1 (Table 1). In soybean seed, galactose is found in arabinogalactan in cotyledons and in galactomannans in seed coats (Morita, 1965a,b; Aspinall et al., 1967). Originally, arabinogalactans in soybean seed were thought to be distinct from the rhamnogalacturonans (Aspinall et al., 1967), which are branched polysaccharides with galacturonic acid backbones (Carpita and Gibeaut, 1993). However, some have suggested that arabinogalactans are branches of rhamnogalacturonans (Carpita and Gibeaut, 1993; Huisman et al., 1999). Rhamnose is a branch point in rhamnogalacturonans for the addition of galactose-, arabinose-, xylose-, and fucose-bearing sidechains (Aspinall et al., 1967). Together, these suggest that most of the variation in PC1 was due to variations in rhamnose and galactans within rhamnogalacturonans. The variation in galactose, rhamnose, and fucose was inversely related to the variation in mannose in PC1. Mannose has been identified in mannans in a hemicellulose fraction and galactomannans in a water-soluble fraction in soybean seed coat (Aspinall et al., 1967). Seed coats contained 49% of mannose isolated in analysis of CWP, but only 6% of the galactose when compared with whole seeds (Stombaugh et al., 2000). Thus, the variation from galactose in PC1 was due to variation in cotyledon CWP, while the variation in mannose could have been due to seed coat or cotyledon CWP. In addition, total fiber analysis did not remove all of the protein from the meal. When additional soybean lines were treated with excess heat-stable protease (type X from *Bacillus thermoproteolyticus* rokko, EC 3.4.24.27) during total fiber analysis to further remove protein, mannose was reduced by 20% or 3.4 g kg^{-1} DM in whole soybean meal. There was no reduction of mannose in seed coat soybean meal following protease treatment (1998, unpublished data). Therefore, the source of variation in mannose that was negatively related to pectin in PC1 could have been due to variation in mannosylated protein within the cotyledon, variation in mannans within the seed coat or cotyledon, or some combination of these.

Principal Components 2 and 3 with contributions

Table 1. Principal components obtained using monosaccharide data of cell wall polysaccharides from 13 Minnesota soybean cultivars averaged across 2 yr and four locations. A correlation matrix was used in the analysis.

Monosaccharide	Principal components			
	1	2	3	4
Galactose	0.906**	-0.308	-0.091	-0.087
Rhamnose	0.823**	0.000	-0.301	0.257
Fucose	0.641*	-0.289	0.285	0.383
Mannose	-0.757**	0.329	0.171	0.196
Arabinose	0.339	0.816**	-0.091	0.123
Glucose	0.375	0.567*	0.563*	0.229
Xylose	0.270	0.509	-0.672*	-0.213
Uronic acids	0.499	0.171	0.558*	-0.600*
% of variance	38.3	19.5	16.2	9.1
Eigenvalues	3.06	1.56	1.30	0.73

* $P < 0.05$.** $P < 0.01$.

from glucose or xylose (Table 1) may describe variation within cellulose, xylans, or xyloglucans. Arabinose varied with PC2 and is confined to that PC. In contrast, uronic acids varied with PC3. Because of the variety of polysaccharides comprised by arabinose, xylose, or glucose, whether PC2 and PC3 describe a particular tissue or polysaccharide type cannot be absolutely determined from these data. In cauliflower (*Brassica oleracea* L. var. *botrytis* L.), pectic compounds were consistently found in association with xylan and xyloglucans (Femenia et al., 1999a,b). Extraction and enzymatic degradation analysis of CWP demonstrated that xylose and arabinose were commonly found in similar polymer fractions in soybean meal (Huisman et al., 1999). Thus, it should not be surprising to observe a pectin constituent such as arabinose varying with xylose or glucose.

Within pectin, arabinose is found in arabinans and in specific ratios with galactose in arabinogalactans purified from soybean cotyledon meal (Morita, 1965a,b; Aspinall and Cottrell, 1971). Arabinose and galactose content were related to cell wall development in several other studies (Nishitani and Masuda, 1979; Kikuchi et al., 1996). The percentage of galactose decreased and the percentage of xylose increased in azuki bean [*Vigna angularis* (Willd.) Ohwi & H. Ohashi] epicotyls as cell wall maturity and distance from the apex increased (Nishitani and Masuda, 1979). Arabinose and galactose varied in the amount attached as sidechains on rhamnogalacturonans in *Daucus carota* L. callus culture (Kikuchi et al., 1996). Functionally, higher Ara:Gal ratios were positively correlated with larger cell clusters and tighter intercellular attachment. Thus, Ara:Gal has been related to cell wall development and function in a number of plants.

Relationships between Cell Wall Polysaccharide and Seed Traits

Cell wall polysaccharides were significantly positively correlated with PC1 as was pectin (Table 2). Neither CWP nor pectin were correlated to any of the other PCs. Thus, monosaccharides significant in PC1 would be responsible for variation seen in total CWP. The correlation between PC1 and protein + oil and the lack of correlation between protein + oil and any of the

Table 2. Correlation coefficients of principal components (PCs) with other seed traits using 13 Minnesota soybean cultivars averaged across 2 yr and four locations.

	PC1	PC2	PC3	PC4
Pectin	0.956**	0.021	0.104	-0.208
CWP†	0.871**	0.339	0.261	-0.084
Seed wt.	0.151	-0.500	-0.377	-0.615*
Yield	0.273	-0.591*	-0.055	0.034
Maturity	0.348	-0.521	-0.084	0.115
Oil	-0.040	0.022	0.269	0.026
Protein	-0.381	-0.185	-0.309	-0.271
Protein + oil	-0.576*	-0.252	-0.262	-0.372

* $P < 0.05$.** $P < 0.01$.

† CWP = cell wall polysaccharide.

other PCs suggest that PC1 describes the variation in CWP responsible for the correlation between protein + oil and CWP observed earlier (Stombaugh et al., 2000). A decrease in these monosaccharides would relate to an increase in protein + oil.

Genotypic variation for yield and maturity was significant for the 13 Minnesota cultivars used in this study (Table 3). Yield exhibited some genotype \times location interaction, but this was a minor portion of the total variation (data not shown). Maturity exhibited no genotype \times environment effects in these studies (data not shown). Yield was significantly negatively correlated with PC2 (Table 2) and positively correlated with maturity (Table 4). Yield and maturity were shown to be correlated previously (Mansur et al., 1996). The negative correlation between yield and PC2 shows that in higher yielding cultivars glucose-containing polysaccharides that vary with arabinose-containing polysaccharides were lower. The polysaccharides or association between polysaccharides that was described by PC2 would be valuable to have quantified as a specific polysaccharide or as quantities of polysaccharides in association with each other to demonstrate whether they will reproducibly show a correlation with yield.

Stepwise regression of the individual monosaccharides against yield produced a model with only arabinose and galactose being significant (Table 5). Galactose and arabinose represented the highest values in PC1 and PC2, respectively (Table 1), and they were not correlated in these data ($r = 0.02$). The significance of these two monosaccharides is more easily shown by calculating

Table 3. Soybean seed yield traits for 13 cultivars averaged across 2 yr and four locations.

Cultivar	Yield	Maturity
	kg ha ⁻¹	d
Parker	3376a†	21.8b
Bert	3197ab	24.6a
Lambert	3140bc	14.1d
Granite	3125bc	25.4a
Hendricks	3108bc	15.1d
Kato	2991bcd	18.3c
Faribault	2965bcd	23.8a
Council	2921cd	11.8e
Toyopro	2800de	14.4d
Ozzie	2633e	8.5f
Proto	2373f	11.0e
Glacier	2372f	2.5g
McCall	2112g	0.4h

† Means within a column and followed by same letter are not significantly different by LSD ($P = 0.05$).

Table 4. Correlation coefficients of monosaccharides from cell wall polysaccharides with other traits using 13 Minnesota soybean cultivars averaged across 2 yr and four locations.

	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acids	Ara:Gal†
Yield	0.298	0.262	−0.520	−0.148	−0.367	0.474	−0.133	−0.039	−0.704**
Maturity	0.403	0.391	−0.386	−0.086	−0.413	0.419	−0.134	−0.013	−0.592*
Seed wt.	−0.017	−0.044	−0.259	0.121	−0.539	0.368	−0.663*	0.135	−0.450
	Yield	Maturity	Seed wt.	Oil	Protein	Protein + oil			
Maturity	0.850**								
Seed wt.	0.216	0.147							
Oil	0.382	−0.002	−0.199						
Protein	−0.336	−0.168	0.281	−0.785**					
Protein + oil	−0.223	−0.243	0.269	−0.451	0.907**				
CWP‡	0.062	0.073	−0.106	0.078	−0.444	−0.587*			

* $P < 0.05$.** $P < 0.01$.

† Ara:Gal = Arabinose:Galactose.

‡ CWP = cell wall polysaccharide.

Table 5. Stepwise regression of yield against arabinose, fucose, rhamnose, galactose, glucose, xylose, mannose, and uronic acids and stepwise regression of seed weight against principal components (PCs); $n = 13$.

	Step	RSQ†	MS	t	Variable	Coefficient	SE	P
Yield								
Constant	1	0.00	31.4		Constant	72.7	36.5	0.07
Arabinose	2	0.27	25.0	−2.0	Arabinose	−4.0	1.7	0.04
Arabinose + galactose	3	0.51	18.6	2.0	Galactose	1.2	0.6	0.05
Adjusted R^2		0.41						
Seed weight								
Constant	1	0.00	5.4		Constant	17.7	0.36	0.00
PC4	2	0.38	3.8	−2.6	PC2	−0.9	0.30	0.01
PC4 + PC2	3	0.63	2.4	−2.6	PC3	0.8	0.32	0.04
PC4 + PC2 + PC3	4	0.77	1.6	2.4	PC4	−1.7	0.43	0.00
Adjusted R^2		0.69						

a ratio of arabinose to galactose. Across cultivars, Ara:Gal varied significantly from 0.47 to 0.59 ($LSD_{0.05} = 0.02$) and 45% of the variability as defined by total mean squares was genotypic (Table 6). Genotypic variation in arabinose and galactose was 21 and 25% of the total mean squares, respectively (Stombaugh et al., 2000), suggesting that Ara:Gal was under tighter genotypic control than the amounts of the individual monosaccharides. Thus, the variation of branching within arabinogalactans across cultivars was significant and large enough for arabinose and galactose to appear uncorrelated; or a significant part of the arabinose was in simple arabinans or another type of polysaccharide not associated with galactose.

The amounts of arabinose and galactose in a soybean cotyledon change during development (Sasaki, 1983; Koch et al., 1999). In one study, the proportion of galactose in CWP remained unchanged while the proportion of arabinose increased (Koch et al., 1999) during seed development. There was a significant increase in arabi-

nose between 38 and 48 d after flowering. During this period, fresh weight peaked, dry weight had almost reached its maximum, and cotyledon yellowing due to maturation commenced. In another study, the proportion of galactose increased and the proportion of arabinose and xylose decreased with increasing cotyledon age in cotyledons harvested simultaneously across developmental stages (Sasaki, 1983). In either case (Sasaki, 1983; Koch et al., 1999), the composition of polysaccharide produced changed during development, suggesting that the final arabinose and galactose content would be related to cell wall development up to the point of dry down. It has not been shown to what extent the change during development in arabinose to galactose was due to genotypic variation or what significance changes over development may have on yield and other seed traits.

When relating yield, maturity, Ara:Gal, and cell wall development in soybean seed, Ara:Gal was negatively correlated with yield and maturity (Table 4) with longer maturing, higher-yielding cultivars having lower Ara:Gal ratios. It is unknown to what extent maturity is associated with the length of seed development in these cultivars. If there is a set developmental pathway for CWP in soybean seed with cell walls later in development having higher Ara:Gal, then cultivars having higher Ara:Gal would have developed farther along this pathway. This would be consistent if higher-yielding cultivars have a shorter seed development phase. However, more data would be needed to test whether CWP change during development vary across cultivars and whether Ara:Gal has a direct affect on yield or is simply an indicator of cell wall development.

Table 6. Mean squares for the ratio of arabinose:galactose (Ara:Gal).

	df	Ara:Gal	MS
Genotype (G)	12		0.0114**
Location (L)	3		0.0030**
Year (Y)	1		0.0061**
G × L	36		0.0005
G × Y	12		0.0006
L × Y	3		0.0034**
G × L × Y	36		0.0005

** Differences significant at the 0.01 probability level.

Seed Weight

Regressing seed traits on the PCs using stepwise regression provided an equation for seed weight (Table 5) with the other traits being insignificant. Principal Components 2, 3, and 4 combined to form an equation with an R^2 of 0.69 for seed weight (Table 5). In Table 4, glucose was correlated with seed weight while the other monosaccharides were not. However, PCA recombined the monosaccharide variation into components that when used in regression analysis achieved a greater correlation between monosaccharides and seed weight (Table 4).

Seed size was shown to be positively correlated with cell number (Guldan and Brun, 1985) and seed growth rate was positively correlated with seed weight and cotyledon cell number (Munier-Jolain and Ney, 1998). The number of cells created during the cell division phase of development is a strong indicator of final seed weight. Cells would need some minimal amount of cell wall material to form primary cell walls. Beyond that, the amount of thickening or secondary wall may vary. Regression of seed weight against the PCs suggests that PC 2, 3, and 4 describe CWP that are involved in determining seed weight (Table 5). Pectin components described by PC1, which are major determinants in total CWP, were not correlated with seed weight (Table 2) or significant in the regression of seed weight against the PCs (Table 5). Principal Component 4 with its strong uronic acid component was correlated with seed weight (Table 2) and was used in the regression equation (Table 5). Since the uronic acids are the backbone units of pectin, and PC1 seems to describe variation of galactans, the galactan portion of pectin was not related to variation in seed weight. Arabinose is considered a pectin component, yet it has been found in close association with xylans and xyloglucans and has been suggested to play a role in cross-linking cell wall fibers (Femenia et al., 1999a,b; Huisman et al., 1999). Therefore, the equation suggests that mostly linear portions of CWP are correlated with seed weight, and uronic acids and glucose content are the most important (Tables 2, 4, and 6).

CONCLUSIONS

Four PCs described relationships between soybean seed monosaccharides and accounted for 83.1% of the monosaccharide variation. Principal component analysis provided a means to demonstrate which monosaccharides varied similarly. From these analyses, we were able to evaluate the amount and type of variation within pectin, hemicellulose, and cellulose. The relationship between seed weight and CWP was described by a regression equation that used PC2, PC3, and PC4. The absence of PC1 from the seed weight equation suggests that galactose monosaccharides in pectin were not a determinant in seed weight, whereas cellulose, hemicellulose, and pectin backbones were involved in determining seed weight.

Galactose and arabinose were the most significant monosaccharides in PC1 and PC2, respectively, and

were significant in a stepwise regression equation for yield. Further, Ara:Gal was negatively correlated with yield, and maturity and may be an indicator of the average stage of cell wall development for a cultivar at maturity. Determining whether Ara:Gal is developmentally regulated and whether deliberately altering the ratio would change yield and maturity would demonstrate what role these CWP have in determining yield and maturity.

Principal Component 1 was negatively correlated with protein + oil and PC2 was negatively correlated with yield. Principal Component 1 described a galactose and rhamnose-containing portion of pectin while PC2 described part of the cellulose or hemicellulose variability and an arabinose-containing component. Reduction of most pectin monosaccharides within PC1 and the significant monosaccharides within PC2 were associated with increased oil, protein, and yield. The results indicate that further reductions in the content of seed CWP or alterations in composition may be beneficial to improving seed traits.

REFERENCES

- Ahmed, A.E.R., and J.M. Labavitch. 1977. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem.* 1:361–365.
- Analytical Software. 1992. Statistix. Version 4.0. Analytical Software, St. Paul, MN.
- Aspinall, G.O., R. Begbie, and J.E. McKay. 1967. Polysaccharide components of soybeans. *Cereal Sci. Today* 12:223–228, 260–261.
- Aspinall, G.O., and I.W. Cottrell. 1971. Polysaccharides of soybeans. VI. Neutral polysaccharides from cotyledon meal. *Can. J. Chem.* 49:1019–1022.
- Brown, J.S. 1991. Principal component and cluster analyses of cotton cultivar variability across the U.S. cotton belt. *Crop Sci.* 31:915–922.
- Carpita, N.C., and D.M. Gibeau. 1993. Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3:1–3.
- Champ, M., J. Brillouet, and X. Rouau. 1986. Nonstarchy polysaccharides of *Phaseolus vulgaris*, *Lens esculenta*, and *Cicer arietinum* seeds. *J. Agric. Food Chem.* 34:326–329.
- Chatterjee, S., and B. Price. 1977. Regression analysis by example. John Wiley and Sons, New York.
- Chen, L., N.C. Carpita, W.-D. Reiter, R.H. Wilson, C. Jeffries, and M.C. McCann. 1998. A rapid method to screen for cell-wall mutants using discriminant analysis of Fourier transform infrared spectra. *Plant J.* 16:385–392.
- Cober, E.R., and H.D. Voldeng. 2000. Developing high-protein, high-yield soybean populations and lines. *Crop Sci.* 40:39–42.
- Daveby, Y.D., and P. Aman. 1993. Chemical composition of certain dehulled legume seeds and their hulls with special reference to carbohydrates. *Swed. J. Agric. Res.* 23:133–139.
- Femenia, A., N.M. Rigby, R.R. Selvendran, and K.W. Waldron. 1999b. Investigation of the occurrence of pectic-xylan-xyloglucan complexes in the cell walls of cauliflower stem tissues. *Carbohydr. Polym.* 39:151–164.
- Femenia, A., K.W. Waldron, J.A. Robertson, and R.R. Selvendran. 1999a. Compositional and structural modification of the cell wall of cauliflower (*Brassica oleracea* L. var *botrytis*) during tissue development and plant maturation. *Carbohydr. Polym.* 39:101–108.
- Guldan, S.J., and W.A. Brun. 1985. Relationship of cotyledon cell number and seed respiration to soybean seed growth. *Crop Sci.* 25:815–819.
- Huisman, M.M.H., H.A. Schols, and A.G.J. Voragen. 1999. Enzymatic degradation of cell wall polysaccharides from soybean meal. *Carbohydr. Polym.* 38:299–307.
- Jung, H.G., and D.R. Buxton. 1994. Forage quality variation among

- maize inbreds: Relationships of cell-wall composition and in-vitro degradability for stem internodes. *J. Sci. Food Agric.* 66:313–322.
- Kikuchi, A., Y. Edashige, T. Ishii, T. Fujii, and S. Satoh. 1996. Variations in the structure of neutral sugar chains in the pectic polysaccharides of morphologically different carrot calli and correlations with the size of cell clusters. *Planta* 198:634–639.
- Koch, J.L., M. Horbowicz, and R.L. Obendorf. 1999. Methanol, pectin and pectinesterase changes during soybean seed maturation. *Seed Sci. Res.* 9:311–320.
- Mansur, L.M., J.H. Orf, K. Chase, T. Jarvik, P.B. Cregan, and K.G. Lark. 1996. Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Sci.* 36:1327–1336.
- Morita, M. 1965a. Polysaccharides of soybean seeds Part I. Polysaccharide constituents of “hot-water-extract” fraction of soybean seeds and an arabinogalactan as its major component. *Agric. Biol. Chem.* 29:564–573.
- Morita, M. 1965b. Polysaccharides of soybean seeds Part II. A methylated arabinogalactan isolated from methylated product of “hot-water-extract” fraction of soybean seed polysaccharides. *Agric. Biol. Chem.* 29:626–630.
- Munier-Jolain, N.G., and B. Ney. 1998. Seed growth rate in grain legumes II. Seed growth rate depends on cotyledon cell number. *J. Exp. Bot.* 49:1971–1976.
- Nishitani, K., and Y. Masuda. 1979. Growth and cell wall changes in azuki bean epicotyls I. Changes in wall polysaccharides during intact growth. *Plant Cell Physiol.* 20:63–74.
- SAS Institute. 2000. SAS user's guide. Version 8. SAS Inst., Cary, NC.
- Sasaki, K. 1983. Changes in neutral sugar compositions of cell wall polysaccharides during redifferentiation of cultured carrot cells and during maturation of soybean seeds. *Plant Cell Physiol.* 24:811–821.
- Stevens, J. 1986. Applied multivariate statistics for the social sciences. Lawrence Erlbaum Assoc., Hillsdale, NJ.
- Stombaugh, S.K., H.G. Jung, J.H. Orf, and D.A. Somers. 2000. Genotypic and environmental variation in soybean seed cell wall polysaccharides. *Crop Sci.* 40:408–412.
- Theander, O., P. Aman, E. Westerlund, R. Andersson, and D. Pettersson. 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and klason lignin (the Uppsala method): Collaborative study. *J. AOAC Int.* 78:1020–1044.